Notes

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Karyotype of Tetraploid *Hieracium alpinum* (Asteraceae)

四倍体チシマタンポポ（キク科）の核型（佐藤杏子[a]，山崎貴博[b]，岩坪美兼[c]）

Summary: The karyotype and meiotic chromosome configuration of a *Hieracium alpinum* cultivated in Japan was studied. This plant was an allotetraploid plant containing inversion and reciprocal translocation chromosomes.

Hieracium L. (Asteraceae) is a genus distributed in Europe, Asia, North and South America, and South Africa (Ohwi and Kitagawa 1992). It consists of approximately 90 species and is subdivided into about 1,000 microspecies (Mabberley 1997). This is a monobasic genus with x = 9 chromosomes (Darlington and Wylie 1955, Fedorov 1969). Its diploids are sexual, and its polyploids are apomicts (Mabberley 1997). *Hieracium alpinum* L. (alpine hawkweed) is distributed from Greenland through central Europe and northwest Russia (Chrtek and Plakèová 2005) and is cultivated as an ornamental plant in Japan. The majority of chromosome counts reported for *H. alpinum* were triploids with 2n = 27 (Table 1), though a hypotriploid plant with 2n = 26 was found in the Ural Mountain region of Russia (Sokolovskaya and Strelkova 1960). Recently, diploid plants with 2n = 18 were found in the Ukrainian region of the Carpathian Mountains (Chrtek 1997), and a tetraploid plant with 2n = 36 was also found in the Tatra Mountains of Poland (Szelag and Jankun 1997).

Karyotypic and meiotic chromosome behaviour analyses are useful methods to elucidate genomic constitutions in polyploid plants. Using these techniques, the karyotype and meiotic chromosome configuration of *H. alpinum* cultivated in Japan was first studied.

The *Hieracium alpinum* plant used for this study was maintained in the experimental garden at the Faculty of Science, University of Toyama. The origin of this particular plant is unknown. Newly formed roots were pretreated in about 2.1 mM 8-hydroxyquinoline solution at room temperature (about 25°C) for one to 1.5 hours, and then maintained at approximately 5°C for 15 hrs. The root tips were prepared in a mixture of glacial acetic acid and absolute ethyl alcohol (1:3) at room temperature for one hr, and were macerated

Table 1. Previous reports for chromosome numbers of *Hieracium alpinum*

2n	Previous reports
18	Chrtek (1997, as *H. alpinum* var. *alpinum*,and *H. alpinum* var. *subglabrum*)
26	Sokolovskaya and Strelkova (1960)
27	Rosenberg (1926a, b), Böcher and Larsen (1950 sec. Fedorov 1969), Skalińska et al. (1959), Skawinska (1962), Uhríková and Murín (1970), Engelskjoen and Knaben (1971), Gadella and Kliphuis (1971), Lavrenko et al. (1988, 1989), Huber and Baltisberger (1992), Měsíček (1992, sec. Goldblatt and Johnson 2000), Murín and Májovský (1992, sec. Goldblatt and Johnson 1996), Chrtek (1994), Chrtek (1997, as *H. alpinum* var. *alpinum*), Stace et al. (1995, sec. Goldblatt and Johnson 1998), Stace et al. (1997), Schuhwerk and Lippert (1999, as *H. alpinum* subsp. *alpinum* and *H. alpinum* subsp. *halleri*), Chrtek et al. (2004), Chrtek and Plačková (2005)
36	Szelag and Jankun (1997)

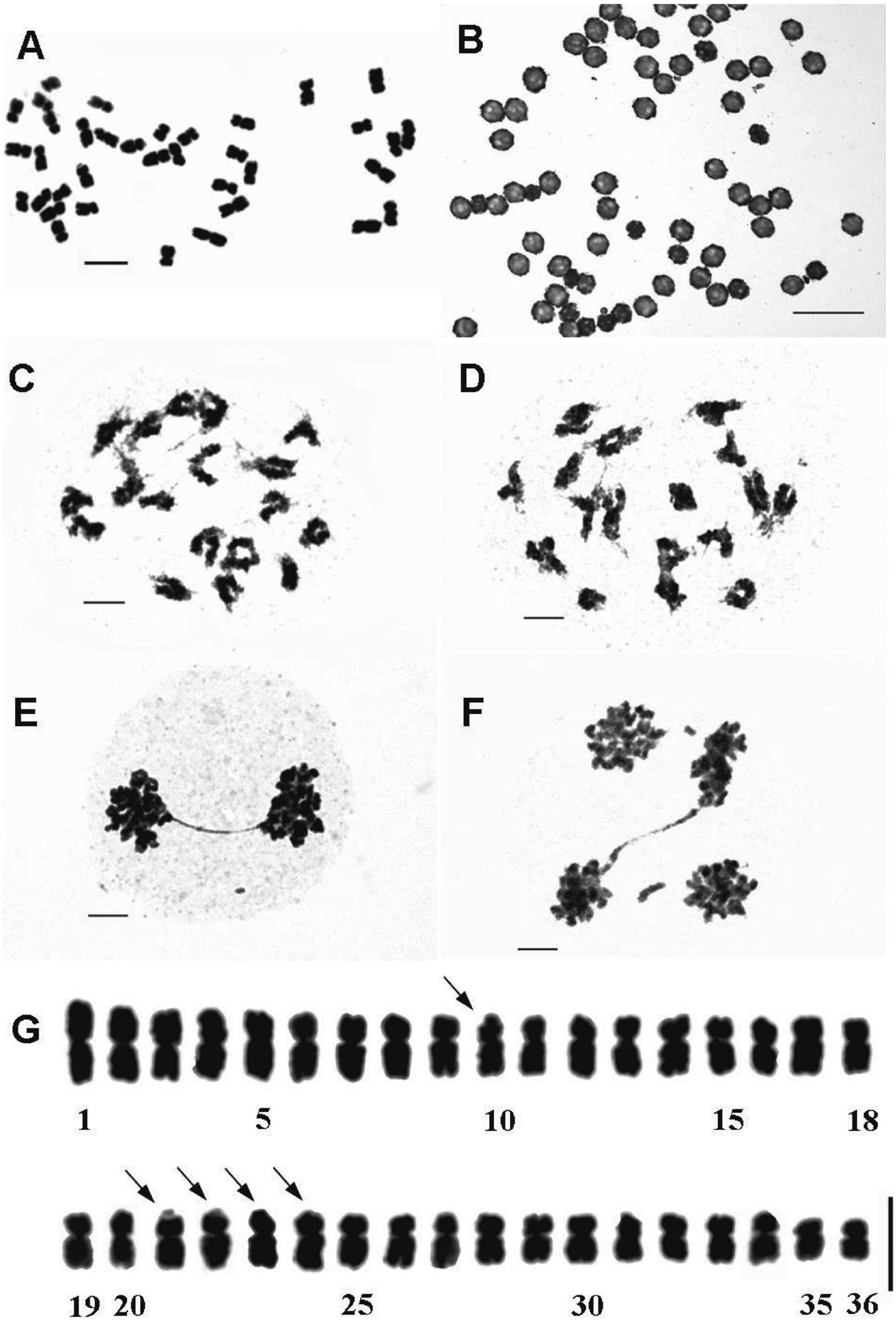

Fig. 1. Chromosomes and pollen grains of a *Hieracium alpinum* plant cultivated in Japan. A. Mitotic metaphase chromosomes. B. Pollen grains. C–D. Meiotic metaphase I. E. Meiotic anaphase I with single chromosome bridge. F. Meiotic anaphase II with single chromosome bridge and laggards. G. Karyogram at mitotic metaphase. Arrows indicate chromosome arms with secondary constrictions. Scale bars represent 5 μm (A, C–G) and 100 μm (B).

Table 2. Measurements of somatic chromosomes of *Hieracium alpinum*

No.	Length (μm)	Total (μm)	A.R.	Form
1	2.0 + 2.1	4.1	1.1	m
2	1.7 + 1.9	3.6	1.1	m
3	1.7 + 1.8	3.5	1.1	m
4	1.7 + 1.8	3.5	1.1	m
5	1.4 + 1.9	3.3	1.4	m
6	1.4 + 1.9	3.3	1.4	m
7	1.4 + 1.9	3.3	1.4	m
8	1.2 + 1.9	3.1	1.6	m
9	1.2 + 1.9	3.1	1.6	m
10	*0.4–0.7 + 1.8	2.9	1.6	m
11	1.2 + 1.7	2.9	1.4	m
12	1.1 + 1.7	2.8	1.5	m
13	1.1 + 1.7	2.8	1.5	m
14	1.1 + 1.7	2.8	1.5	m
15	1.1 + 1.7	2.8	1.5	m
16	1.1 + 1.7	2.8	1.5	m
17	1.1 + 1.7	2.8	1.5	m
18	1.1 + 1.6	2.7	1.5	m
19	1.1 + 1.5	2.6	1.4	m
20	1.1 + 1.5	2.6	1.4	m
21	t-1.0 + 1.6	2.6	1.6	m
22	t-1.0 + 1.6	2.6	1.6	m
23	t-1.0 + 1.6	2.6	1.6	m
24	t-1.0 + 1.6	2.6	1.6	m
25	1.0 + 1.5	2.5	1.5	m
26	0.8 + 1.5	2.3	1.9	sm
27	0.8 + 1.5	2.3	1.9	sm
28	1.0 + 1.3	2.3	1.3	m
29	0.9 + 1.3	2.2	1.4	m
30	0.9 + 1.3	2.2	1.4	m
31	0.9 + 1.3	2.2	1.4	m
32	0.9 + 1.3	2.2	1.4	m
33	0.9 + 1.3	2.2	1.4	m
34	0.9 + 1.2	2.1	1.3	m
35	0.8 + 1.0	1.8	1.3	m
36	0.8 + 1.0	1.8	1.3	m

*satellite.
t: small satellite.

in 1N hydrochloric acid at 60°C for 10 min, then washed in tap water; they were subsequently stained and squashed in 1.5% lacto-propionic orcein. A cell with fully spread metaphase chromosomes was used for describing its chromosomal form according to the nomenclature of Levan et al. (1964).

For the study of meiotic chromosome pairing, young flower buds were prepared in Newcomer's fluid at 17°C for three hrs; the buds and anthers were macerated, stained and squashed using the same procedure previously done with the root tips. The chromosome pairing was studied in pollen mother cells; pollen grains harvested from flowerheads in full bloom and fixed in 70% ethanol were stained in 1.5% lacto-propionic orcein. To determine the percentage of stained pollen grains as a possible indicator of pollen fertility, 1,739 pollen grains were examined under a light microscope. Voucher specimen is kept at the Toyama Science Museum (TOYA).

Concurring with the study of Szelag and Jankun (1997), this plant was found to be a tetraploid with $2n = 36$ chromosomes (Fig. 1A), with somatic metaphase chromosomes ranging between 1.8 μm to 4.1 μm in length and an arm ratio of 1.1 to 1.9 with a gradual and symmetric karyotype (Table 2). The chromosomes were divided into two groups constituting 34 metacentric chromosomes and two submetacentric chromosomes (Fig. 1G). In the somatic complement, five chromosomes within the metacentric ones had satellites on their short arms; within five satellite chromosomes, one arm was longer than the others. Within the chromosome complement, the two smallest chromosomes were metacentric with a 1.3 arm ratio measuring 1.8 μm in length. They were easily distinguished from the others by their remarkably smaller sizes and were considered as a homologous pair.

The pollen fertility level was 67.1 percent (Fig. 1B), with the pollen mother cells showing abnormal microsporogenesis in the form of a tetravalent formation with chromosome bridges and chromosome fragments. In meiotic metaphase I (Figs. 1C, 1D), there were either only bivalents, or a mixture of bivalents and one tetravalent. Chromosome bridges were found in anaphases I (Fig. 1E) and II (Fig. 1F), with chromosome laggards found only at anaphase II.

The appearance of chromosome bridges demonstrates that this plant is an inversion heterozygote, with the existence of a tetravalent indicating a reciprocal translocation included in the chromosome complement. Such karyotypic features suggest that this plant is an allotetraploid plant that includes inversion and reciprocal translocation chromosomes, with the complement composed of one pair of chromosome sets containing two smaller chromosomes.

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栽培されていたチシマタンポポ *Hieracium alpinum* について染色体の観察を行ったところ，染色体数は2n = 36であり，染色体基本数 x = 9 に基づく四倍体であった．核型分析を行ったところ，染色体の長さは1.8～4.1 µm，腕比は1.1～1.9であり，５本の染色体の短腕にサテライトがみられた．核型は中部動原体型染色体と次中部動原体型染色体からなる勾配的かつ対称的核型であった．最小の一組の中部動原体型染色体は，他と比較して明らかに小型であった．花粉母細胞の減数分裂を観察したところ，二価染色体のほかに四価染色体が認められた．第一分裂後期および第二分裂後期においては染色体橋がみられ，第二分裂後期では遅滞染色体も観察された．花粉の染色性とサイズにおいて，正常な花粉は67.1%だけであった．以上の結果から，今回観察を行ったチシマタンポポは，逆位と相互転座を含む異質四倍体であると判断される．

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Kazumi FUJIKAWA[a], Htum Paw OO[b], Aung DIN[c], Kyaw KHAING[d] and Minoru OKADA[a]: A Botanical Inventory in Myanmar (1). Market Research of Herbal Medicine

Summary: Since 2000, the Makino Botanical Garden has been conducting plant inventory research in Myanmar, based on a Memorandum of Understanding signed by the Forest Department, Ministry of Forestry of Myanmar and the Makino Botanical Garden. As a part of the inventory program, the authors conducted a survey of herbal medicines on sale at local markets in order to gauge the extent of use of basic plant resources. The contents of a total of 181 herbal medicines purchased at the markets were confirmed by interviewing people at the point of sale and the staff of Forest Department and by cross-referencing with floras and other books. A preliminary checklist to all the plant species contained in the products is presented here, and detailed descriptions (including local names, uses, prices and photos) of six particularly